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Homeobox genes are transcriptional transregulators that control the expression of multiple genes responsible for embryogenesis, morphogenesis, and cell differentiation. The fact that altered expression and/or mutations of homeogenes have been found in human malignancies, such as leukemias and solid tumors in lung, stomach, colon, kidney, testis and breast, indicates that these genes may constitute a new class of protooncogenes. The expression of homeogenes in normal human breast tissues and their alteration in breast cancer, in particular, suggest that these genes may play a role in the normal development and physiology of the breast. Human chorionic gonadotropin (hCG) has been found to be an efficacious physiological protector in rats against the initiation and progression of mammary tumors induced by carcinogen, accompanied by the induction of cellular differentiation and expression of the α and β forms of inhibins in mammary gland *in vivo* and *in vitro*. As inhibins are activated by hCG in mammary tissue, it is likely that hCG, homeogenes, and inhibins are connected to one another in a common pathway controlling cellular differentiation. **We hypothesize that hCG may induce cellular differentiation, through activating and/or maintaining the normal expression of homeogenes that in turn promote the expression of genes responsible for differentiation.**

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Principal Investigator's Signature

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Homeobox Genes Mediate the Biological Functions of Human Chorionic Gonadotropin (hCG) in Human Breast Epithelial Cells

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5-INTRODUCTION

Subject

Homeobox genes (homeogenes) are transcriptional factors controlling the expression of multiple genes implicated in embryonic development and differentiation. The temporal activation of homeogenes is colinear with the patterns of expression in the body, determining the body pattern and cell identity. The deregulation of this temporal linearity is thought to produce congenital anomalies (1). The involvement of homeogenes in adult tissues has been documented by their regulatory roles in normal and neoplastic hematopoiesis (2-4). Homeogenes detected in adult tissues have been found to be deregulated in solid cancers of kidney, testis, prostate, stomach, colon, and breast (5-8). These findings indicate that homeogenes may also play an important role in maintaining the normal physiology of cells and their abnormalities may contribute to the carcinogenesis processes. Several peptide growth factors of a cystine-knot family, including transforming growth factor (TGF), activin, platelet derived growth factor (PDGF) and fibroblast growth factor (FGF), have been shown to activate the expression of homeogenes (9-11). However, there is no definitive evidence of how homeogenes are regulated by these growth factors and whether they mediate their ultimate biological roles.

Our laboratory is interested in elucidating the mechanisms through which human chorionic gonadotropin (hCG) affects cell differentiation and mammary cancer prevention(12). The molecular structure of hCG reveals a cystine-knot folding motif that is found in peptide growth factors known to activate the expression of homeogenes. In addition, activins, dimeric glycoproteins composed of βA or βB subunits of inhibin, are found to be co-expressed with homeogenes in several tissues. As inhibins are activated by hCG in mammary tissue, it is likely that hCG, homeogenes, and inhibins are connected to one another in a common pathway controlling cellular differentiation.

Purpose

Based on these data, we hypothesize that hCG may induce cellular differentiation through activating and/or maintaining the normal expression of homeogenes that in turn promote the expression of genes responsible for differentiation. For this purpose, we have proposed to identify specific homeogenes that are expressed either normally or only activated by hCG in human breast epithelial cells *in vitro*, and further to determine whether those homeogenes activated by hCG have direct effects on the expression of cellular differentiation genes of these cells.

The relevance of this study lies in the concept that hCG may behave as a developmental factor or primer inductor of differentiation by activation of homeogenes that when activated trigger a cascade of gene activation. Further relevance of this concept is the fact that hCG is a protective factor in breast carcinogenesis, therefore hCG may be the driving force of the well known protective effect of early pregnancy and the preventive role in rat mammary carcinogenesis.

The knowledge gained with these studies will have significant impact in our understanding of the regulation of homeogenes by hCG, and furthermore will open a new horizon in the utilization of hCG for inducing breast differentiation and cancer prevention.

Scope of research

Homeobox genes (homeogenes) are transcriptional factors controlling the expression of multiple genes responsible for embryo development and differentiation. They share a highly conserved 183 bp region encoding a 61 amino acid DNA-binding domain called "homeodomain". Initially discovered in Drosophila as homeobox complex (HOM-C), homeogenes have been identified in essentially all animal species, including nematodes and vertebrates (13-15). Human homeogenes are organized into four clusters (HOXA, B, C and D) that are respectively located on chromosomes 7, 17, 12, and 2 (Fig 1), in addition to the so-called divergent homeogenes that are not distributed in these clusters but widely in the genome (16,17). The temporal activation of homeogenes is colinear with the patterns of expression in the body, determining the body pattern and cell identity; the deregulation of this temporal linearity is thought to produce congenital anomalies (1). The involvement of homeogenes in adult tissues has been well documented by their regulatory roles in normal and neoplastic hematopoiesis (2-4). In addition, homeogenes have been detected in adult tissues and found to be de-regulated in solid cancers of kidney, testis, prostate, stomach, colon and breast (5-8). These findings have indicated that homeogenes may also play an important role in maintaining the normal physiology of cells,while their abnormalities may contribute to carcinogenic processes.

The regulation of the homeobox genes is poorly understood. In Drosophila the Polycomb-group (Pc-G) genes and the Trithorax (*trx*) genes have been respectively identified as epigenetic upstream negative and positive regulators of the HOX-C genes through inducing heterochromatin formation or promoting opened chromatin structure (4). In vertebrates, the Pc-G homolog *bmi*-1 gene was shown to cause posterior skeletal transformation, impaired lympho-myelopoiesis, and to promote the development of lymphomas in *Eμ-myc* transgenic mice (reviewed in 4). Retinoic acid (RA) and several peptide growth factors such as TGF, activin, PDGF and FGF, on the other hand, have been shown to activate the expression of homeogenes (9-11). RA activates the expression of homeogenes in a sequential order colinear with the 3'-5' localization in the clusters. RA excess or mutations of its receptor, similar to the overexpression of Hox genes, induces teratogenic development of the vertebrate embryos (18). The direct regulation of homeogenes by RA is supported by recent evidence for the presence of functional RA response elements (RARES) in Hoxa-1, Hoxb-1 and Hoxd-4 genes (18). Morphogenetic proteins (BMPs), the TGFβ homologs, activate the expression of homeogenes Mxs-1 (19) and Abd-B (20), acting as inductive signals between tissue layers in the embryo. Activins (a homodimer of β-strain inhibins) are co-expressed with homeogenes during morphogenesis (21), and found to activate homeogene Gnot1 (22). These findings have demonstrated that these peptide growth factors and probably other members of the cystine knot superfamily, such as human chorionic gonadotropin (hCG), luteinizing hormone (LH) and follicle stimulating hormone (FSH) (23), may represent potential regulators controlling the expression of homeogenes. Further studies of these factors in the regulation of homeogenes will provide insight into the mechanisms of embryo development, cellular differentiation and pathogenesis of malignancies. There is evidence that the homeobox genes are involved in the development and

growth of the mammary glands and that the expression of these genes may be altered during mammary carcinogenesis. For example, the Hoxc-6 gene is expressed in pubescent and mature mammary glands, but its expression is decreased in pregnancy and in precancerous tissue, and lost in mouse mammary tumors. In contrast, the Hoxa-1 gene is not expressed in normal or precancerous mammary tissues, but only in mammary tumors (24). A close relationship in the expression level of divergent homeobox genes Msx-1 and Msx-2 with mouse mammary development is also well documented (25). In breast cancer-derived MCF-7 cells HOXA1, HOXB6, HOXA10 and HOXC6 transcripts have been detected (16,17). These findings support the notion that HOX genes may represent a novel class of protooncogenes. Furthermore, in collaborative work with Raman et al. (26) we have demonstrated that some homeobox genes, such as HOXA5, are expressed only in normal breast tissues and breast epithelial cells, but not in breast cancers and cancer-derived cells, an expression pattern consistent with that of a tumor suppressor gene. hCG has been explored as a potential physiological factor for breast cancer prevention based upon its stimulatory effect on the differentiation of the virgin rat mammary gland, namely since the induction of this phenomenon is as efficacious as a full term pregnancy in inhibiting the initiation and progression of mammary tumors induced by the carcinogen 7,12-dimethylbenz(a)anthracene (12). Classically, hCG is known to interact with a G-protein coupled lutropin-choriogonadotropin receptor (LH-CG-R), activating adenylyl cyclase and increasing the production of cAMP, which leads to increased synthesis of estrogen, progesterone and inhibins (reviewed in 12). However, little is known about the mechanism(s) through which this hormone induces differentiation of the mammary gland that result in protection against mammary carcinogenesis. **Based on the notion that cystine-knot growth factors may regulate the expression of homeogenes (11) and the fact that hCG belongs to the cystine-knot family (27), we proposed to test the hypothesis that hCG may induce cellular differentiation through activating and/or maintaining the normal expression of homeogenes that in turn promote the expression of genes responsible for differentiation.**

We have previously shown that hCG inhibits *in vitro* cell proliferation of the spontaneously immortalized human breast epithelial cell line MCF-10F and of the breast cancer cells MCF-7, with a phenomenon accompanied by the induction of synthesis of α and β chains of inhibins. This phenomenon parallels *in vivo* findings demonstrating that mammary differentiation induced by hCG in virgin rats also results in increased synthesis of inhibin (12). Furthermore, in collaborative work with Raman et al. (26), we have found that the homeogene HOXA5 is down-regulated or lost in breast cancer cells MCF-7. These human breast epithelial cells are providing an *in vitro* experimental model for us to test first whether hCG regulates the expression of homeogenes, and to further determine their role in mammary cell differentiation. These experiments should generate valuable information on the role of homeogenes as mediators for action of hCG, and more importantly novel research targets for breast cancer prevention through physiological approaches.

6-BODY

Experimental Methods and Procedures

We have proposed three specific aims: 1-To identify and characterize homeogenes that are

expressed in the immortalized human breast epithelial MCF-10F cells and in the breast cancer-cell line MCF-7, 2-To determine the patterns of expression levels of homeogenes in the above cells after treatment with hCG at various times and doses, and 3-To determine if hCG-activated homeogenes can directly activate genes indicative of cell differentiation of human breast epithelial cells

During this first year of the grant award we have concentrated in the first two aims. In these two aims we have utilized reverse transcription-polymerase chain reaction (RT-PCR) to generate homeodomain-containing cDNA using degenerate primer sets specific for the homeodomain helix 1 and 3 sequences, as indicated in 6-E and in Table 1.

A. **Reagents and treatment regimens:** In the original application we proposed to utilize commercially available human chorionic gonadotropins that have been obtained from the urine of pregnant women, such as hCG from Sigma (St Louis, MO), Wyeth-Ayerst (Montreal, Canada), NIDDK (Bethesda, MD), or Organon (Oss, Netherlands). These preparations are less than optimal due to the presence of contaminant pregnancy-related peptides and growth factors that rest specificity to the assays performed. For that reason we have utilized instead recombinant hCG provided by Serono Laboratories (Geneva, Switzerland). The advantage of this preparation is its high purity and freedom from other hormonal residues. We initially proposed to use the following concentrations of hCG in the culture medium: 1, 10, 50, 100 and 1000 IU for 24 h treatment. We found that the optima biological response of gene induction was obtained with 5 μg r-hCG (amount equivalent to 100 IU), therefore all the experiments were performed using this dose. We had initially proposed the following durations of hCG treatment at an optimal dosage: 0, 24, 48, 72, 96, 120 and 144 hours. However, we observed that critical changes in gene expression were seen at much earlier times, therefore we have changed this protocol to study earlier points, such as 1, 5, and 10 hours for comparison with effects seen at 24, 48 and 96 hours.

B. **Source and maintenance of cells:** The spontaneously immortalized human breast epithelial cells MCF-10F, which originated from the mortal cells MCF-10M, are maintained in our laboratory. MCF-7, a breast carcinoma cell line, was purchased from the American Tissue Type Collection (Rockville, MD). MCF-10F cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM)/F-12 (1:1), supplemented with equine serum, insulin, hydrocortisone, epidermal growth factor, cholera toxin, and antibiotics. The cells were cultured at 37^0C in a humidified atmosphere under 5% CO_2.

C. **Analysis of Gene Expression:** The cell lines listed above were plated in six T150 flasks (Falcon, Lincoln Park, NJ) concentration of $1x10^4$ cells per cm^2. When the cells reached 80% confluence they were treated with r-hCG (Serono, Geneva, Switzerland). Cells were treated for periods of time ranging from 1, 5, 10, 24, 48, and 96 hours, with daily changes of the hormone-containing medium. Control cells were treated with the same volume of buffer in which r-hCG was dissolved. The cells were harvested at the end of each treatment period and immediately frozen in liquid nitrogen for RNA extraction. RNA was isolated from all the treated and control cells described above using Trizol Reagent according the manufacturers specifications (Gibco BRL, Rockville, MD).

D. RT-PCR was performed by using QIAGEN OneStep RT-PCR Kit and 800 ng of total RNA was used for each reaction. Reverse transcriptions were performed at 50^0C for 35 min. PCR were carried out starting with a 15 min at 95^0C followed by a denaturation step at 94^0C for 45 sec, an annealing step at the appropriate temperature for 45 sec and an extension step at 72^0C for 45 sec for 40 cycles. Each reaction was finished by a final extension run at 72^0C for 10 min. Specific primers for each gene of the clusters A, B, C and D were used as reported in Table 1 (see Addendum-B). Semi-quantitative analysis of the PCR products for the expression levels was carried out comparing the controls without hCG treatment by means of Computing Densitometer (Molecular Dynamics, USA). The ratio of sample: control at each time spot was analyzed.

E. The following 39 Class I human Hox gene genes were sequenced at the Fannie E. Rippel Biotechnology Facility of the Fox Chase Cancer Center: HOXA1-HOXA13, HOXB1-HOXB13, HOXC4-HOXC13, HOXD1, HOXD3, HOXD4, and HOXD8-HOXD13. Homeobox clusters are shown according to their physical position on the chromosomes (Table 1).

Results

a. Identification of homeobox genes that are expressed in the immortalized human breast epithelial cells MCF-10F and in the breast cancer cell line MCF-7.

Comparison of the 39 Class I human Hox gene expression in MCF-10F and MCF-7 cells are depicted in Figure 1 (See addendum B). MCF-10F and MCF-7 cells expressed 35 and 38 out of the 39-homeobox genes tested, respectively. The expression of HOXA2, B7, B13 and C10 was not detected in MCF-10F cells. HOXA2 was the only silent HOX gene in MCF-7 cells.

b. Effect of r-hCG treatment on the pattern of expression of homeobox genes inMCF-10F and MCF-7 cells.

Treatment of MCF-10F and MCF-7 cells with r-hCG affected the level of expression of the following homeobox genes (Figures 2 and 3 in Appendix B). In MCF-10F cells r-hCG induced at 48 hours of treatment downregulation of HOXA1, and HOXB3, and upregulation of HOXA6, HOXC8, and HOXD8 (Figure 2, Appendix B). HOXB4, HOXB5, and HOXC13 were slightly upregulated, but the rate was not significant. (Figure 2, Appendix B). In the case of HOXB3, marked downregulation was observed at 24 hours of treatment, and the HOXA6 is downregulated after 1 hour of treatment, returning to normal levels thereafter (Figure 4, Appendix B). (These data clearly indicated that the hormone may influence the level of activation of several Hox genes and that timing is important in their activation process. For example HOXA6 is activated very early after initiation of treatment, whereas other genes require longer period of time. Another important aspect is that whereas some Hox genes are down regulated other, like HOXC8 and HOXD8 are upregulated. The biological significance of these changes is the objective of specific aim 3.

In the human breast cancer cell line MCF-7, the action of r-hCG at 48 hours of treatment downregulated HOXB3 and upregulated HOXB8, HOXC10, and HOXD8 (Figure 3, Appendix B). HOXB3

Discussion

Hox genes encode transcription factors that provide positional information during morphogenesis along the body axis. Genetic interaction among *Hox* genes is thought to be necessary for correct pattern formation. One of the most curious features of the 39 vertebrate *Hox* genes is that they form four clusters each composed of several genes paralogous between clusters (33). Homeobox-containing genes encode transcriptional regulators involved in cell fate and pattern formation during embryogenesis. Recently, it has become clear that their expression in continuously developing adult tissues, as well as in tumorigenesis, may be of equal importance. A growing number of data have sustained the involvement of homeobox gene expression deregulation in cancer. In this study, we have performed an exhaustive survey of the expression of the 39 class I *Hox* genes in MCF-10F and MCF-7 cells. We were not able to detect the expression of HOXA2, HOXB7, HOXB13 and HOXC10 in MCF-10F cells. In contrast, there was only one silent HOX gene (HOXA2) in MCF-7 cells. Ours is the first study of the Hox serie I genes in these two cell lines. Our results coincide with similar observations reporting that the vast majority (34-39) of HOX genes is expressed in normal and malignant human cervical keratinocytes, hematopoietic cells, and colonic cells.

Our results demonstrated that HOXB3 was down regulated by r-hCG treatment of both MCF-10F and MCF-7 cells. In endothelial cells (EC) that express several members of the Homeobox (Hox) gene family, suggesting a role for these morphoregulatory mediators during angiogenesis, HOXB3, influences angiogenic behavior in a manner that is distinct from HOXD3. The paralogous gene HOXD3 is required for expression of integrin alpha-beta3 and urokinase plasminogen activator (uPA), which contributes to EC adhesion, invasion, and migration during angiogenesis. The antisense to the paralogous gene HOXB3 impaired capillary morphogenesis of dermal microvascular EC cultured on basement membrane extracellular matrices. Although levels of HOXD3-dependent genes were maintained in these cells, levels of the ephrin A1 ligand were markedly attenuated. Capillary morphogenesis could be restored, however, by addition of recombinant ephrin A1/Fc fusion proteins. To test the impact of HOXB3 on angiogenesis in vivo, this geneHOXB3 was constitutively expressed in the chick chorioallantoic membrane using avian retroviruses that resulted in an increase in vascular density and angiogenesis. Thus, while HOXD3 promotes the invasive or migratory behavior of EC, HOXB3 is required for the subsequent capillary morphogenesis of these new vascular sprouts. Together, these results support the hypothesis that paralogous Hox genes perform complementary functions within a particular tissue type. (37). In the case of MCF-7 and MCF-10F cells the downregulation of HOXB3 gene may be paralogous with other genes such as HOXA1, HOXB4, HOXB5, HOXC8, and HOXC13, by r-hCG and may have relevance to the protective effect of hCG *in vitro* by altering basic molecular mechanism that may interfere with the cells to elicit and angiogenic response. This needs further studies.

Treatment of MCF-10F cells with r-hCG upregulates the expression ofHOXC8. HOXC8 acts as a transcriptional corepressor, inhibiting bone morphogenetic protein (BMP) signaling in the

nucleus when interacting with Smad6. . Smad6 and Smad7, a subgroup of Smad proteins, antagonize the signals elicited by TGFβ. These two Smads, induced by TGFβ or BMP stimulation, form stable associations with their activated type I receptors, blocking phosphorylation of receptor-regulated Smads in the cytoplasm (38). Additional research is needed for clarifying how HOXC8 interacts with Smads under the inlfunce of r-hCG. Another important function of Hox C has been described in mixed lineage leukemia (MLL), which is frequently involved in chromosomal translocations associated with acute leukemia and is the one established mammalian homologue of Trx. Bmi-1, first identified as a collaborator in c-myc-induced murineF lymphomagenesis and is homologous to the Drosophila Pc-G member Posterior sex combs (39). Embryonic fibroblasts from MLL-deficient compared with Bmi-1-deficient mice demonstrate reciprocal regulation of Hox genes as well as an integrated HOXC8-lacZ reporter construct. Reexpression of MLL was able to overcome repression, rescuing expression of HOXC8-lacZ in MLL deficient cells (39). A further role in the differentiation of neural tube has been attributed to HOXC8 (40). Of interest is the fact that overexpression of a HOXC8 transgene causes cartilage defects whose severity depends on transgene dosage. The abnormal cartilage is characterized by an accumulation of proliferating chondrocytes and reduced maturation (41). It remains to be investigated whether the upregulation of HOXC8 gene by r-hCG treatment of human breast epithelial cells reflects characteristics specific to the breast epithelium, since it is not known whether these oncogenes interact similarly in cells from different tissue of origin. These possibilities need to be explored further.

Hox A1 is downregulated in MCF-10F cells by r-hCG. It is of interest that in the mouse mammary epithelial cell lines SCp2 and CID-9 HOXA-1 expression is downregulated when the cells are cultured on an inert substratum, but not when an grown on an adequate extracellular matrix (42).

HOX B4 and HOXB5 are essentially unchanged by r-hCG treatment of human breast epithelial cells. In contrast, cultured lung cells treated with EGF, TGF-β 1, and EGF + TGF-β1 exhibit a 20-70% downregulation in the expression of HOXB5 (43).

All together our data indicate that r-hCG is a trigger of homeobox changes that may explain the differentiation effects of this hormone in breast epithelia.

7-KEY RESEARCH ACCOMPLISHMENTS

- MCF-10F and MCF-7 cells expressed 35 and 38 out of the 39-homeobox genes tested, respectively.
- The expression of HOXA2, B7, B13 and C10 was not detected in MCF-10F cells.
- HOXA2 was silent in MCF-7 cells.
- In MCF-10F cells r-hCG induced at 48 hours of treatment downregulation of HOXA1, and HOXB3, and upregulation of HOXA6, HOXC8, and HOXD8. HOXB4, HOXB5, and HOXC13 were slightly upregulated, but the rate was not significant.
- In the human breast cancer cell line MCF-7, the action of r-hCG at 48 hours of treatment downregulated HOXB3 and upregulated HOXB8, HOXC10, and HOXD8.

8-REPORTABLE OUTCOMES

None

9-CONCLUSIONS

We have clearly demonstrated that an immortalized human breast epithelial cells MCF-10F, that has all the characteristics of a normal epithelial cell, and the breast cancer cells MCF-7 express most the Hox A, B, C and D genes. This is the first report that shows with the highly sensitive RT-PCR that these genes are not silent in the breast epithelium. Of relevance is the fact that these Hox genes are responding to the *in vitro* action of r-hCG. The promissory data collected emphasize the need of more extensive studies of the role of these Hox genes as transcription factors for the expression of genes with more defined biological properties.

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"Homeobox Genes Mediate the Biological Functions of Human Chorionic Gonadotropin (hCG) in Human Breast Epithelial Cells"

11-APPENDICES

"Homeobox Genes Mediate the Biological Functions of Human Chorionic Gonadotropin (hCG) in Human Breast Epithelial Cells"

Appendix A. Acronyms and Symbol Definition

[S^{35}] α-dATP	S^{35} labeled deoxyadenotriphosphate
Abd-B	a divergent homeobox gene
BMPs	Morphogenetic proteins
cAMP	cyclic adenosine monophoph
CAT	chloramphenicol acetyltransferase
CD-9	a cell differentiation gene
cDNA	complementary deoxynucleotide acid
DC-59	a cell differentiation gene
dNTP	deoxynucleotide triphosphate
Eμ-myc	an engineered version of oncogene myc
FGF	fibroblast growth factor
FSH	follicle stimulating hormone
Gnot1	a divergent homeobox gene
hCG	human chorionic gonadotropin
HD	homeodomain
HOM-C	homeobox complex
HOXA, B, C, D	four clusters of homeobox genes in human
Hoxa, b, c, d	four clusters of homeobox genes in vertebrates
IU	international unit
LH	luteinizing hormone
LH-CG-R	lutropin choriogonadotropin receptor
MCF-10F	spontaneously immortalized human breast epithelial cell line
MCF-7	human breast cancer derived epithelial cell line
Msx-1	a divergent homeobox gene isolated from mouse mammary tissue
Msx-2	a divergent homeobox gene isolated from mouse mammary tissue
Na^+/K^+-ATPase α	sodium-potassium adenosin triphosphorase alpha
Pc-G	the Polycomb-group genes in fruit fly
pCMV	transient expression plasmid vector
PDGF	platelet derived growth factor
pT7 RNA 28S	primer set specific for ribosomal 28S RNA
pTRI-GAPDH	primer set specific for glyceraldehyde-3-phosphate dehydrogenase
pTRI-β-actin-125	primer set for a 125 base pair actin
RA	retinoic acids
RARES	retinoic acid response elements
RNA	ribonucleotide acid
RT-PCR	reverse transcription-polymerase chain reaction
TGF	transforming growth factor
trx	Trithorax genes

"Homeobox Genes Mediate the Biological Functions of Human Chorionic Gonadotropin (hCG) in Human Breast Epithelial Cells"

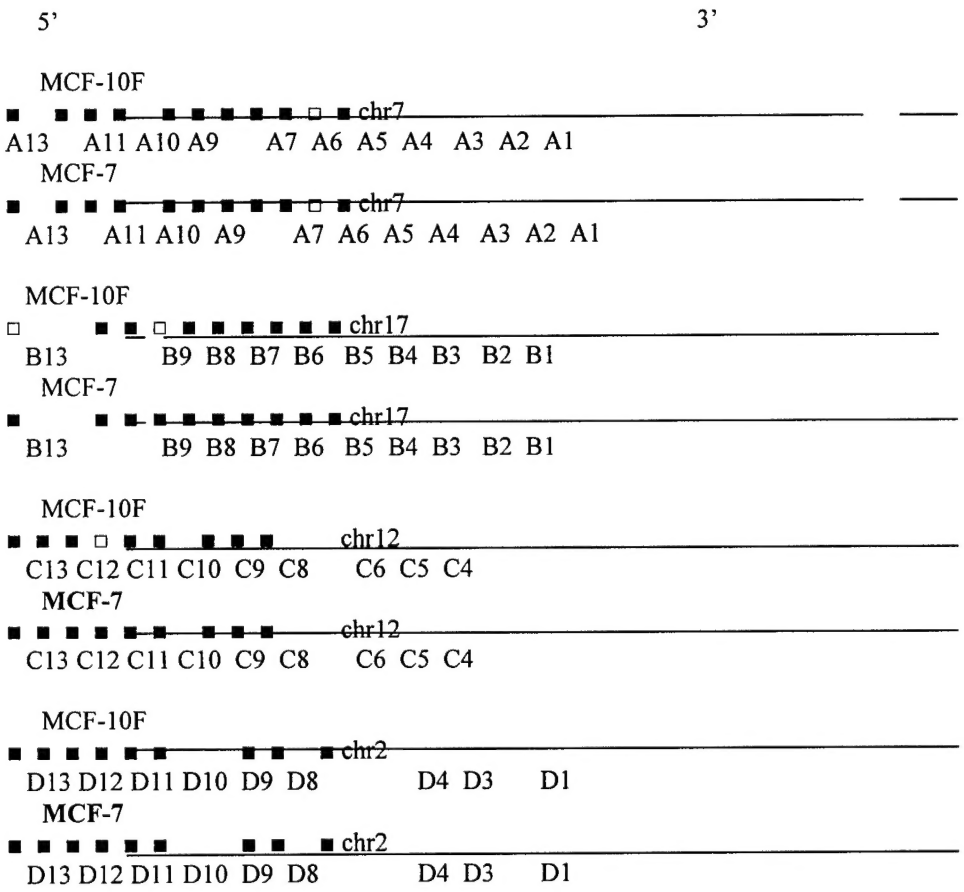
Appendix B. Illustration/Diagrams/Chemical Synthesis

Table 1: Gene specific primers for analysis of HOX genes expression by RT-PCR

Gene	5' Primer	3' Primer	T^0C	Size(bp)
HOXA1	5' ATGAACTCCTTCCTGGAATA 3'	5' CGTACTCTCCAACTTTCC 3'	57	452; 655
HOXA2	5'ATCCCTGGATGAAGGAGAAGAAGGC3'	5'ATTGGGAGCCTGCTGTTGAGAGAGC3'		60 487
HOXA3	5'GAAGAATTCAGGAAGCGCC3'	5'TTCCAGCAACCAAGATTGC3'	56	223
HOXA4	5'TGTACCCCTGGATGAAGAAGATCC3'	5'CATTCTCCGGTTCTGAAACCAGATC3'		60 224
HOXA5	5'TGCGCAAGCTGCACATAAGTCATG3'	5'TTGAAGTGGAACTCCTTCTCCAGC3'		60 112
HOXA6	5'GATGCAGCGCATGAACTCCTGCG3'	5'TGGGCTGCGTGGAATTGATGAGC3'	60	250
HOXA7	5'GATCTGGAGAAGGAGTTCCA3'	5'CTTTCTTCCACTTCATACGA3'	55	134
HOXA9	5'CAGCCAACTGGCTTCATGCG3'	5'CACTCGTCTTTTGCTCGGTC3'	55	229
HOXA10	5'AGAGCAGCAAAGCCTCGCCGGAGAAG3'	5'GGACGCTGCGGCTAATCTCTAGGCG3'		65 198
HOXA11	5'ACCCGCAAAAAGCGCTGC3'	5'GAGCTAGCAGGACAGTTG3'	55	114
HOXA13	5'GGGAGAAAGAAGCGCGTG3'	5'CGTCGTGGCTGATATCCG3'	55	114
HOXB1	5'CCTTCTTAGAGTACCCACTCTG3'	5'GCATCTCCAGCTGCCTCCTT3'	55	826
HOXB2	5'TCCTCCTTTCGAGCAAACCTTCC3'	5'AGTGGAATTCCTTCTCCAGTTCC3'		55 353
HOXB3	5'AGTACAAGAAGGACCAGAAGGC3'	5'TGGAGTGTTAAGGCGTTCATG3'		60 120
HOXB4	5'GTGCAAAGAGCCCGTCGTCTACC3'	5'CGTGTCAGGTAGCGGTTGTAGTG3'		60 161
HOXB5	5'GTTCCACTTCAACCGCTACC3'	5'TGTCCTTCTTCCACTTCATGC3'	58	122
HOXB6	5'GGCGAGGCCGCCAGACATAC3'	5'ACTCGGCCTGTTTTTCTTCC3'		55 236

HOXB7	5'AGAGTAACTTCCGGATCTA3'	5'TCTGCTTCAGCCCTGTCTT3'	55	274
HOXB8	5'AGCCTCCTTGTGCAATTG3'	5'GTAACAATTGCCCACAGC3'	54	300
HOXB9	5'GAGCAGGGCAAAGAGTAA3'	5'CTTTCTCCTGACACCTAG3'	54	250
HOXB13	5'CTGGAACAGCCAGATGTGTT3'	5'TTGGCGAGAACCTTCTTCTC3'	60	300
HOXC4	5'CAGTATAGCTGCACCAGTCTCCAGG3'	5'GATCTGCCTCTCAGAGAGGCACAG3'	60	406
HOXC5	5'TGGATGACCAAACAGCACATGAGC3'	5'CAAGTTGTTGGCGATCTCTATGCG3'	60	149
HOXC6	5'CACCTTAGGACATAACACACAGACC3'	5'CACTTCATCCGGCGGTTCTGGAACC3'	60	317
HOXC8	5'CCACGTCCAAGACTTCTTCCACGGC3'	5'CACTTCATCCTTCGATTCTGAAACC3'	58	449
HOXC9	5'TGGTTTCAGACCCGGAGGATCAAG3'	5'GGAAGAGAACGCAGTTTCTCTCC3'	58	436
HOXC10	5'CTACCGCCTGGAACAACCTGTTGG3'	5'ATGGTCTTGCTAATCTCCAGGCG3'	58	662
HOXC11	5'AGAAGCTCGTGCCCTTATTCG3'	5'ATACTGCAGCCGGTCTCTGC3'	57	191
HOXC12	5'AAAGAAGGCGCAAGCCGTATTCGAAG3'	5'AGACGTTGCTCCCTCAGCAGAAGTC3'	58	192
HOXC13	5'TGCCCTATACCAAGGTGCAG3'	5'TAGATTTGCTGACCACCTTT3'	60	173
HOXD1	5'AATCTGGTTCCAGAACCGC3'	5'ATACAAGCTTGCATGCCTGC3'	58	198
HOXD3	5'CATCAGCAAGCAGATCTTCC3'	5'AGCGGTTGAAGTGGAATTCC3'	58	187
HOXD4	5'TGGATGAAGAAGGTGCACG3'	5'ACTTCATCCTCCGGTTCTGG3'	58	220
HOXD8	5'AATCAGCAGCTCCTGGTCGACG3'	5'TGTCTTCCTCCAGCTCTTGG3'	56	279
HOXD9	5'GATGAGCAAGGAGAAATGCC3'	5'AGTCGCTGGAGAGTTTCTGG3'	56	104

HOXD10 5'TACTCCAACCAGCAATTGGC3' 233	5'CTCGGATTCGATTCTCTCGG3'	57
HOXD11 5'ATGCTCAACCTCACTGACCG3' 230	5'AGTTCCACAGAGAAGAGGCG3'	58
HOXD12 5'CCAGCTTCAAGGAAGACACC3' 333	5'CCGCTTCTTCTTCATACGC3'	60
HOXD13 5'AGAATGGAGAACGAGTATGCC3' 114	5'CGGTTCTGAAACCAAATGG3'	55



□ unexpressed HOX gene
■ expressed HOX gene

Fig.1. Diagram of HOX gene expression in MCF-10F and MCF-7 cells.

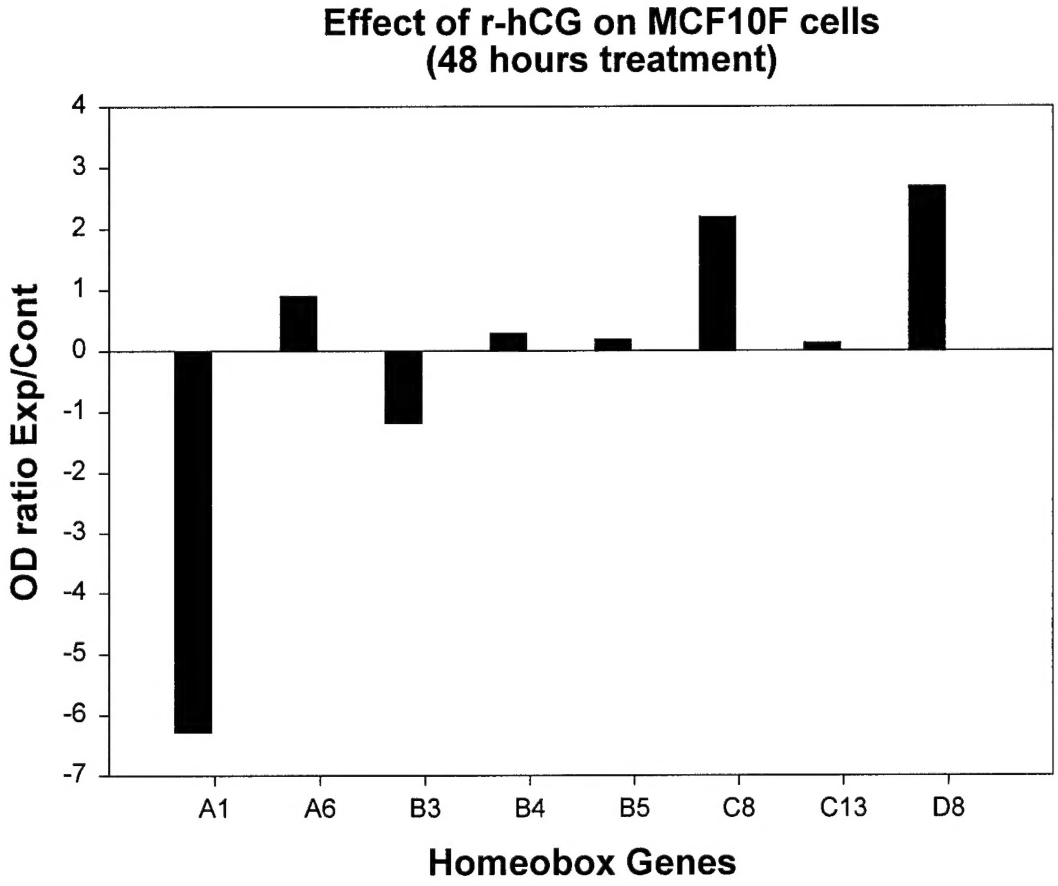


Figure 2: Histogram depicting the activation of HOX genes in MCF-10F treated with 5 μg/ml r-hCG for 48 hours.

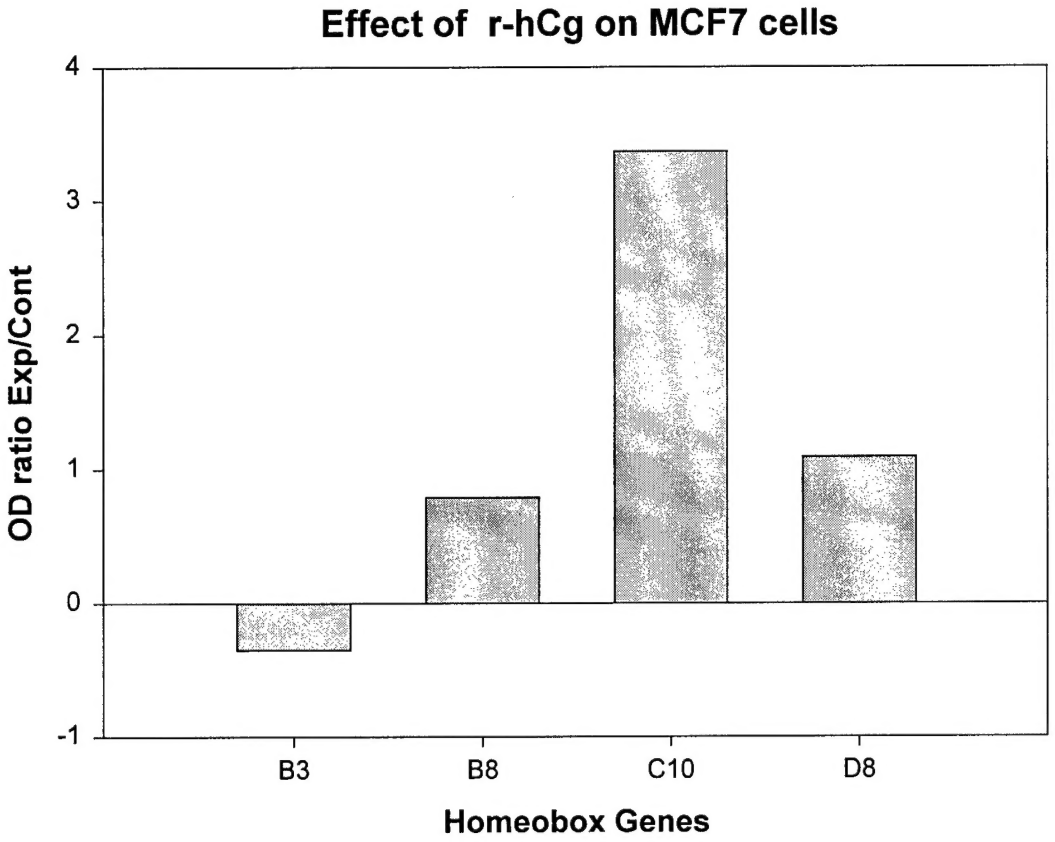


Figure 3: Histogram depicting the activation of HOX genes in MCF-7 treated with r-hCG for 48 hours at a doses of 100IU per ml.

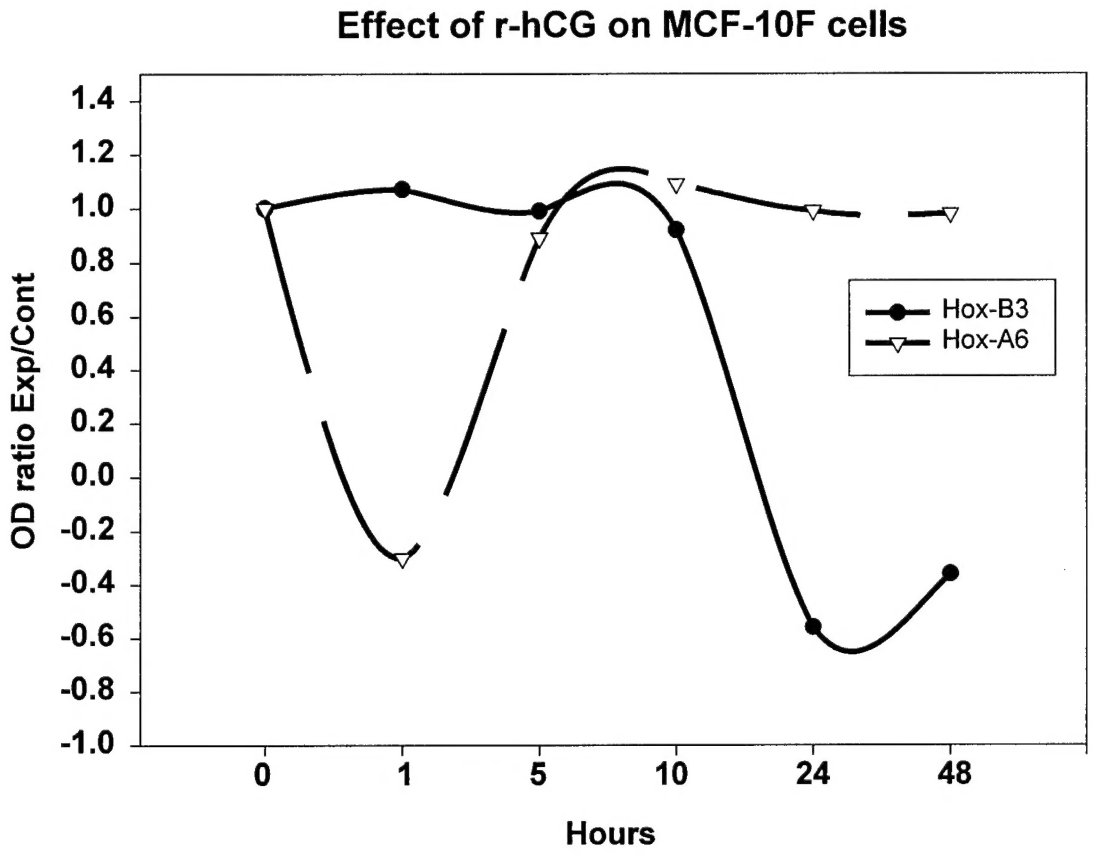


Figure 4: Effect of r-hCG on MCF 10F cells. The HOXB3 and HOXA6 genes were detected by RT-PCR at different times of treatment.